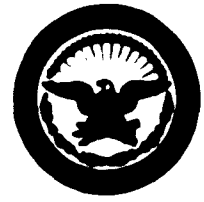

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DETECTION OF MICROBIAL CONTAMINANTS IN JP-4 FUEL

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INTRODUCTION:

The problem of microbiological contamination of JP-4 fuel and fuel systems has been recognized by the Air Force since 1956. Microorganisms have been found actively growing in fuel storage tanks, in integral wing tanks of jet aircraft, and in components of fuel-handling systems. There is strong evidence that microorganisms and/or their metabolic by-products are implicated in the clogging of fuel filters, corrosion of fuel system materials, and actual changes in fuel composition. Adding to the complexity of the problem is the wide variety of types of organisms which have been found growing in fuel samples. These include some common aerobic bacteria, sulfate-reducing bacteria, iron-depositing bacteria, strict and facultative anaerobic bacteria, several genera of filamentous fungi, yeasts, and other microorganisms which cannot be classified.

One of the difficult aspects in the study and control of these microorganisms is the detection of the living cells in fuel before the appearance of the physical results of their metabolic activity, such as sludge or slime formation. Another problem is the differentiation of living cells from dead cells, other organic and inorganic contaminants. Since it is not possible to use standard biological methods for enumerating or detecting organisms or for growth studies without either altering the metabolic patterns or actually killing the organism, we decided that a technique that would measure a metabolic by-product of living cells would be most desirable. This technique also must meet the requirements of simplicity, specificity, and possible applicability in the field. Since all living microorganisms produce carbon dioxide as a product of metabolism, a detection method based on measurement of carbon dioxide produced during cellular respiration has been devised. Non-viable cells and other organic and inorganic contaminants and atmospheric CO_2 levels do not interfere with this measurement.

EXPERIMENTAL PROCEDURES:

Glass columns are packed with soda lime containing an indicator and the ends of the columns are sealed with tissue paper. They are placed near the air-fuel interface of a JP-4 sample. The CO_2 produced by the contaminating organisms is absorbed on the soda lime resulting in a color change of the indicator. This change in color of the indicator reflects the presence of living organisms. The time required for a given length of color change is an approximation of the level of growth or activity of cells.

EXPERIMENT 1. This first experiment was designed to establish optimum column size and CO_2 absorbent mesh size. A series of serum bottles containing 8 ml of sterile Bushnell-Haas medium (see Appendix 1) overlaid with 32 ml of sterile JP-4 fuel was inoculated with a fungus (Hormodendrum). Uninoculated sterile controls were set up in a similar manner. Columns 75 mm long with an internal diameter of 1.85 mm, 120 mm long and 2.5 mm internal diameter, and 160 mm long and 4 mm internal diameter were packed with carbon-dioxide-indicating soda lime.* Two particle sizes of the soda lime were used, 60-100 mesh and 160-200 mesh. These columns were suspended approximately 5 mm above the fuel-air interface and the system was sealed with paraffin and incubated at room temperature. An indicator color change occurred in all inoculated samples. However, the most rapid and sensitive color change occurred in the system using columns 75 mm by 1.85 mm. The two mesh sizes of the CO_2 absorbent showed approximately the same color change. All controls were negative. The indicator color change was measured in mm per number of days incubated.

EXPERIMENT 2. In this experiment, a larger volume system was used to determine if a greater ratio of air to CO_2 would decrease the response due to dilution. A battery jar containing 1500 ml of sterile Bushnell-Haas medium overlaid with 3000 ml of sterile JP-4 fuel was inoculated with two 5-ml water bottom samples from Ramey AFB (Tanks 62 and 55). Three types of columns were used: the first was 75 mm long by 1.85 internal diameter packed with "Indicating Soda Lime,"* 160-200 mesh; the second was 75 mm long by 1.85 mm internal diameter packed with "Metalime,"** 60-100 mesh; and the third was 120 mm long by 2.5 mm internal diameter packed with "Indicating Soda Lime,"* 160-200 mesh. These columns were suspended 10 mm above the fuel-air interface and the system sealed and incubated at room temperature. The results indicated the column 1.85 mm internal diameter packed with "Indicating Soda Lime" exhibited the most rapid color change. Results are listed in table 1.

TABLE 1

COLOR CHANGE
(in mm/time in days)

COLUMN	DAYS						
	3	4	5	6	7	10	11
1	6	9	10	13	15	19	23
2	3.5	4.5	5.5	7	8	11	12.5
3	3	4.5	[illegible].5	7	8	11.5	16.5

* Mallinckrodt Company, "Indicating Soda Lime."
** McKesson Appliance Co., Toledo, Ohio.

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On the basis of these two experiments, columns 75 mm long by 1.85 mm internal diameter packed with "Indicating Soda Lime,"* 60-100 mesh, were used in all subsequent experiments. The pH of the Bushnell-Haas phase of this system was determined before inoculation and 6 weeks after inoculation. The pH changed from 6.9 to 6.5 even though the Bushnell-Haas exhibits a buffering action. This pH drop could be due to metabolic by-products such as fatty acids. Further work is being done to verify the presence of this acid formation and determine its possible correlation with corrosion of metals.

EXPERIMENT 9. A series of 4-ounce medicine bottles containing 20 ml of sterile Bushnell-Haas medium overlaid with 60 ml of sterile JP-4 fuel was inoculated with fuel samples from various Air Force installations. Uninoculated sterile controls were set up. Packed columns were suspended approximately 5 mm above the fuel-air interface and the system sealed and incubated at room temperature. The results are listed in table 2.

TABLE 2

COLOR CHANGE
(in mm/time in days)

SAMPLE	DAYS						
	2	5	6	9	12	13	14
1	0	0	1	2	3	3.5	4
2	0	0	2	4	5.5	6	6
3	0	1	3	11	14	17	19
4	1	6	9	18	24	27	28
5	0	0	0	2	6	6.5	8
6	0	8	12	15	24	24	25
7	0	0	0	1	2.5	4	5
8	0	0	0	1	3	4	4.5
9	0	0	0	0	3.5	6	7
10	0	0	0	0	0	0	3
11	0	0	0	0	0	0	0
12	0	0	0	0	0	0	5
13	0	0	0	0	0	0	0
14	0	0	0	0	3.5	5	6
15	0	0	0	0	0	0	0

* Mallinckrodt Company.

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The change in color of the indicator appeared earlier and proceeded most rapidly in samples 3, 4, and 6. These samples also showed visually the most rapid and dense growth at the fuel-Bushnell-Haas interface. In all cases visible growth was preceded by the color change of the indicator. In the samples with no color change there was no visible growth. The controls were uniformly negative.

DISCUSSION:

The technique described has proved successful for the qualitative detection of microorganisms in the laboratory samples of JP-4 fuel and tank water bottoms tested to date. The samples with no microbial contamination have been consistently negative and those with active microbial contamination have shown color change of the indicator in varying amounts. We have been able to correlate roughly the rapidity and length of color change in the columns due to CO_2 production by the organisms with the appearance and density of visible growth. A rough index of microbial growth has been established as any color change in the indicator within 14 days. However, this index is predicated on the system conditions as outlined above, i.e., columns 1.85 mm internal diameter packed with 60-100 mesh "Indicating Soda Lime"* suspended over 80 ml sample in a 4-ounce bottle. Time of reaction under different conditions will vary and must be standardized for given conditions.

Attempts are being made to correlate mm of indicator color change with number of organisms. Methods of quantitation of cells involving determination of total weight or total nitrogen have not proved completely satisfactory. If total weight after filtration through a Millipore filter or after centrifugation is used as an estimation of number of organisms, any non-viable organisms or inorganic and organic contaminants present can contribute an appreciable error. Another problem of quantitation is that total weight or total nitrogen content per weight of samples containing bacteria or fungi or mixed populations are not necessarily comparable since various types of microorganisms vary widely in their composition. Therefore, the determination of total weight or total nitrogen need not necessarily reflect the number of organisms in the fuel.

There is another aspect of this problem which merits consideration. It is certainly possible that the total number of organisms present is not as important as the type of organism. For example, fungi or slime-forming bacteria contributing to mat formation at the fuel-water interface are potentially more dangerous even in small numbers than other types of organisms. It also has been suggested that a "primary invader" organism may first alter the environment sufficiently so that other organisms can exist and grow in the fuel. With this type of symbiotic relationship, the number of organisms loses importance in comparison to the activity of organisms in the fuel. Therefore,

* Mallinckrodt Company.

although work is continuing on quantitatively correlating the number of organisms with the amount or length of color change in the indicator column, the qualitative method of detection is recommended since any type of microorganism can be a potential problem either in itself or living in symbiotic relationship with other organisms.

SUMMARY:

A qualitative method for the detection of living microorganisms in JP-4 fuel based on the measurement of CO_2 produced by cellular respiration is described. Methods of quantitation are being investigated but have not yet proved satisfactory.

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APPENDIX 1

COMPOSITION OF BUSHNELL-HAAS

Magnesium sulfate	0.2	gm
Calcium chloride	0.02	gm
Potassium phosphate, monobasic	1.0	gm
Ammonium nitrate	1.0	gm
Potassium phosphate, dibasic	1.0	gm
Distilled water	1000	ml

Adj to pH 7.0